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# Appropriate timing of blood sampling for blood gas analysis in the ventilated rabbit

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ABSTRACT

*Background:* Arterial and venous blood gas analyses (BGAs) are essential to evaluate devices that measure biological oxygenation. The appropriate timing of blood sampling for BGA after respiratory rate (RR) change in animal experiments has not been reported. This study investigated the appropriate timing of blood sampling for BGA in ventilated rabbits and whether venous samples are an alternative to arterial samples.

*Materials and methods:* Under general anesthesia, 14 rabbits (body weight, 3.02 ± 0.09 kg) were ventilated and their RR was changed (40/min, 30/min, and 20/min). Blood was sampled through cervical arterial and venous catheters. Experiment 1: in seven rabbits, arterial BGA was measured at 0, 0.5, 1, 2, 3, 5, 10, 15, and 20 min after the RR change. Experiment 2: in seven different rabbits, simultaneous arterial and venous BGA were measured at 0, 2, 5, 10, 15, and 20 min after the RR change.

*Results:* Oxygen partial pressure ($PO_2$) and saturation ($SO_2$) of the arterial blood stabilized 0.5 min after the RR changed. In venous BGA, no index stabilized during observation. The arterial and venous values of the carbon dioxide partial pressure ($PCO_2$) and pH had significant correlations (arterial $PCO_2$ = 0.9316 × venous $PCO_2$−4.4425 [$r$ = 0.9178]; arterial pH = 1.0835 × venous pH−0.5795 [$r$ = 0.9453]).

*Conclusions:* In ventilated rabbits, arterial $PO_2$ and $SO_2$ stabilized in 0.5 min. No venous value stabilized after the RR change. Only the $PCO_2$ and pH of venous samples may be an alternative to arterial samples under the defined formula.



## Introduction

The development of new medical devices has driven the progress of intensive medicine.[1] Medical monitoring devices such as the electrocardiograph and pulse oximeter are broadly used to assess a patient's condition noninvasively and to decide whether medical treatments are needed. In clinical settings, the pulse oximeter is widely used. This

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simple device monitors oxygen saturation in arterial blood and provides information about the respiratory state. However, some studies[2–4] show limitations in the accuracy of the pulse oximeter, and blood gas analysis (BGA) is recognized as the most reliable index for respiratory condition.

Pulse oximeter and real-time monitoring of oxygenation using similar technology (i.e., near-infrared spectroscopy [NIRS])[5,6] are also used in intensive care for neonates with congenital or peculiar diseases.[7] Relative to the blood gas analyzer, these devices sometimes cause improper readings because of hemodynamic collapse due to hypothermia, hypoxia, or acute anemia; skin color; and brightness around the sensor. A dramatic change in circulation occurs in neonates because of hypothermia, hypoxia, or subsequent oxygen therapy.[2] In these specific conditions, the accuracy of a pulse oximeter and NIRS may be unreliable.[5,6,8–10] To assess accuracy of these devices, *in vivo* experiments are an important step to prove the utility of these devices, although experiments in humans are sometimes unacceptable for ethical reasons. Therefore, animal experiments remain important.[11]

BGAs in experimental animals are essential to improve oxygenation monitoring systems[11]; however, no study exists concerning the appropriate timing after the change in the settings of a ventilator. In several studies that used pulse oximetry in rabbits,[12–14] arterial blood gas was sampled at 2 min after the setting of artificial ventilation. The investigators confirmed that the values of the pulse oximeter were stabilized at this point, but they did not confirm that the values of arterial blood gas were stabilized.

In human arterial BGA, the time to reach oxygenation equilibrium after changing respiratory conditions is reportedly 7-20 min.[15,16] In general, cardiac output is a linear factor of oxygen delivery in tissues.[17] Edwards *et al.*[18] report that the average cardiac output of adult rabbits weighing 2.0 kg is 350 mL/min. The average human cardiac output in male adults weighing 70 kg is 5 L/min.[19] Rabbits have 2.39 times larger cardiac output per kilogram of body weight. Therefore, we set a hypothesis that the time to reach oxygenation equilibrium in rabbits are 3-8.4 min because of the difference of cardiac output between human and rabbits.

Venous blood gas seemed to be an important factor to discuss oxygenation and oxygen consumption; however, the behavior of venous blood oxygenation is unclear. The effect of arterial and venous blood gas values of tissue oxygen saturation, as measured by NIRS in medical practice, is controversial.[20] Arterial and venous BGA is essential for further research. To the best of our knowledge, there has been no study on simultaneous arterial and venous gas analysis.

In this study, we therefore used rabbits, which have a similar body weight as a human neonate and are widely used as models of acute respiratory disease and validation of pulse oximeters.[12–16,21] The aim of the study was to investigate the appropriate timing of blood sampling for BGA in a ventilated rabbit model and to investigate whether venous samples are an alternative to arterial samples.

## Materials and methods

### *Ethical approval*

All animal experiments were approved by the Animal Experimentation Committee at the National Defense Medical College (Tokorozawa, Saitama, Japan). The approval number is 13091.

### *Animal preparation*

Fourteen adult 15-wk to 21-wk-old female Japanese White rabbits weighing 3.02 ± 0.089 kg (mean ± standard deviation) were used for the experiment.[22] The animals were purchased from a laboratory animal supplier (Kitayama Labes Co, Ltd, Nagano, Japan) and housed singly in a cage in our animal facility more than 1 wk before the experiments to acclimate them to the environment. Constant room temperature (25°C) and humidity (50% ± 5%) were maintained with 12 h of light-dark cycle. They had *ad libitum* access to pellet food and water.

### *Anesthesia and monitoring*

Anesthesia was induced by an intramuscular injection of mixture of 35 mg/kg ketamine (Ketalar; Daiichi Sankyo Co, Ltd, Tokyo, Japan) and 5 mg/kg xylazine (Sederac; Nippon Zenyaku Kogyo Co, Ltd, Fukushima, Japan) in the gluteal region.[23] Figure 1 shows the experimental setup. After the eyelash reflex and pinch reflex disappeared, the rabbits were placed supine on a heating table (KN303-B; Natsume Seisakusho Co, Ltd, Tokyo, Japan), and the hair in the neck and left hindlimb were shaved. All surgical procedures were performed under sterile conditions. The rabbits were monitored by a pulse oximeter, a noninvasive manometer, and an electrocardiograph with three needle-type subcutaneous electrodes (Life Scope BSM-5192; Nihon Kohden Corp, Tokyo, Japan). The subcutaneous electrodes were placed at the right and left upper chest and left upper abdomen. A lead II electrocardiogram monitored the animals throughout the experiment. Blood pressure in the right lower thigh was noninvasively measured every 2.5 min by the manometer (Life Scope BSM-5192; Nihon Kohden Corp). The rabbits' bodies were covered by a heating blanket (Homeothermic Monitor K020917; Harvard Apparatus, Holliston, MA) and their rectal temperature was maintained at 39.5°C ± 1.0°C.[24]

A 23-gauge venous catheter (Surflo, SR-OT2419C; Terumo, Tokyo, Japan) was inserted in the left auricular vein, and physiological saline (Otsuka Pharmaceutical Co, Ltd, Tokushima, Japan) was infused at a rate of 6 mL/kg/h.[25] An L-shaped incision was created in the neck region. A midline incision was made through the jaw to the jugular notch of the sternum and extended 3-cm horizontally to the right. A tracheotomy was performed 1-cm dorsal from the cricoid and a tracheal tube with a 4-mm internal diameter (RUSCH Safety Clear; Teleflex Medical OEM, Gurnee, IL) and a 5.3-mm external diameter was cut to 11 cm length and distal 4 cm of tube was inserted into trachea (Fig. 2A). Tracheal tube was connected to

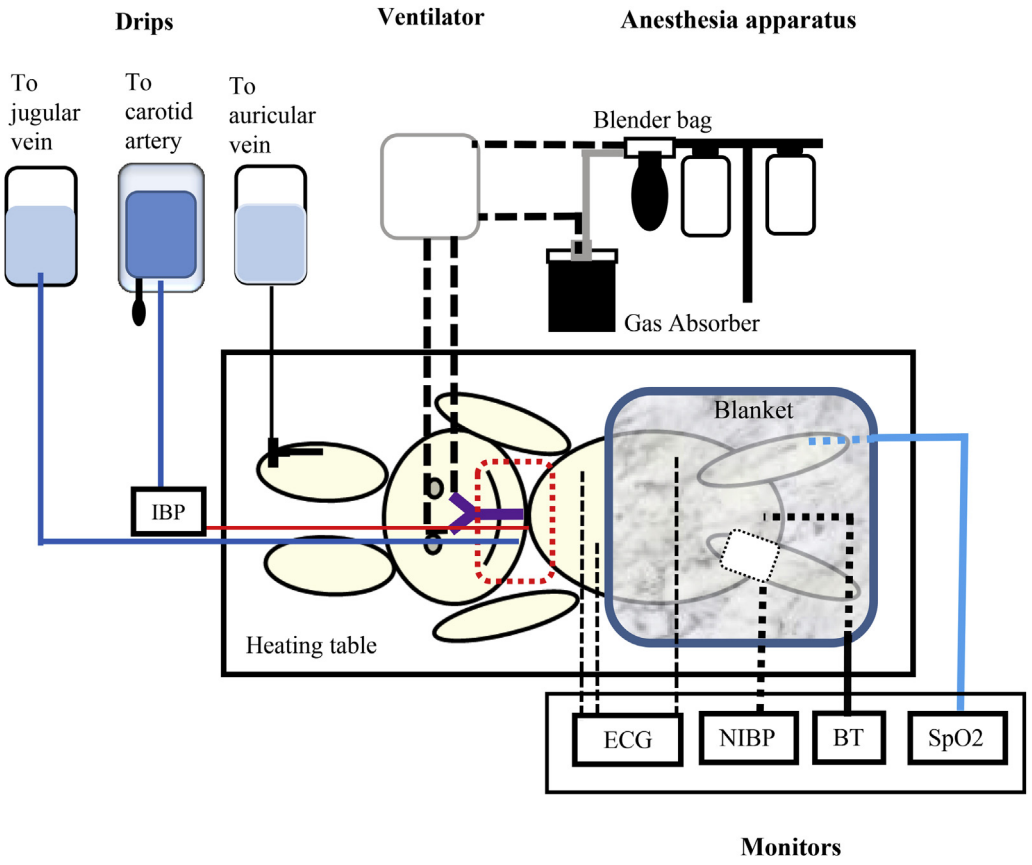


**Fig. 1 – Experimental setup. The cervical area circled by red broken line is enlarged into Figure 2. BP = invasive blood pressure; ECG = electrocardiograph; NIBP = noninvasive blood pressure; BT = body temperature; $SpO_2$ = arterial oxygen saturation measured by pulse oximetry.**

Y-Luer adapter (73-4121, Harvard Apparatus, Holloston, MA). Y-Luer adapter was connected to a ventilator (Volume Control 155-7058; Harvard Apparatus, Holliston, MA) via silicon tubes. All rabbits were artificially ventilated with room air at a respiratory rate (RR) of 40 per min, which was calculated by formula (1),[26,27] assuming that the weight was 3.0 kg at the start of the experiments. The tidal volume was calculated by formula (2).[26,27]

$$\text{Respiration rate/min} = 53.5 \times (\text{body weight})^{-0.26} \quad (1)$$

$$\text{Tidal volume} = 0.0062 \times (\text{body weight})^{1.01} \quad (2)$$

General anesthesia was maintained by 1.0% of sevoflurane (Sebofuren; Maruishi Pharmaceutical, Osaka, Japan) inhalation,[28] and muscular relaxation was maintained with a bolus intravenous infusion of 0.6 mg/kg rocuronium bromide (Eslax, 50 mg/5.0 mL; MSD Co, Tokyo, Japan). An additional 0.6 mg/kg bolus infusion of rocuronium bromide was added when any sign of spontaneous respiration appeared.[29] The concentration of sevoflurane was maintained within 0.5%-2.0% to maintain sufficient anesthesia[28] and minimum blood pressure (i.e., mean arterial pressure >40 mm Hg or systolic pressure >60 mm Hg[30] by noninvasive monometer or invasive monometer by arterial catheter).

### *Arterial and venous catheterization*

Figure 2B shows the surgical view of catheterization procedure. We exposed the right carotid artery and identified the bifurcation of the right carotid artery and the right brachiocephalic artery. We used a microscope (ocular, ×10; magnification,×0.8; Stemi 2000-C; Carl Zeiss Industrielle Messtechnik GmbH, Oberkochen, Germany) to incise the right carotid artery and to insert a 22-gauge catheter (Surflo, SR-OT2232C; Terumo, Tokyo Japan), which connected to a three-way stopcock (R1-L; Top Co, Tokyo, Japan), barotolerance extension tube (S50; Hakko Co, Ltd, Nagano, Japan), barometer transducer (MP333N; Edward Lifesciences Co, Irvine, CA), barometer infuser (Mediquick Plus ME-ACS-223; Terumo, Tokyo, Japan), and a 500-mL saline bottle with 125 units of heparin to avoid coagulation.[31,32] To collect circulating blood, we placed the tip of a catheter at the origin of the right carotid artery from the brachiocephalic artery (i.e., the bifurcation point of the right carotid artery and brachiocephalic artery), as shown in Figure 2B.

We then exposed the right jugular vein running laterally and inserted a 20-gauge catheter (Surflo, SR-OT2032C; Terumo, Tokyo Japan) so that its tip was at the junction of the right jugular and subclavian vein, as shown in Figure 2B. The catheter and the tube were connected to the infusion bottle

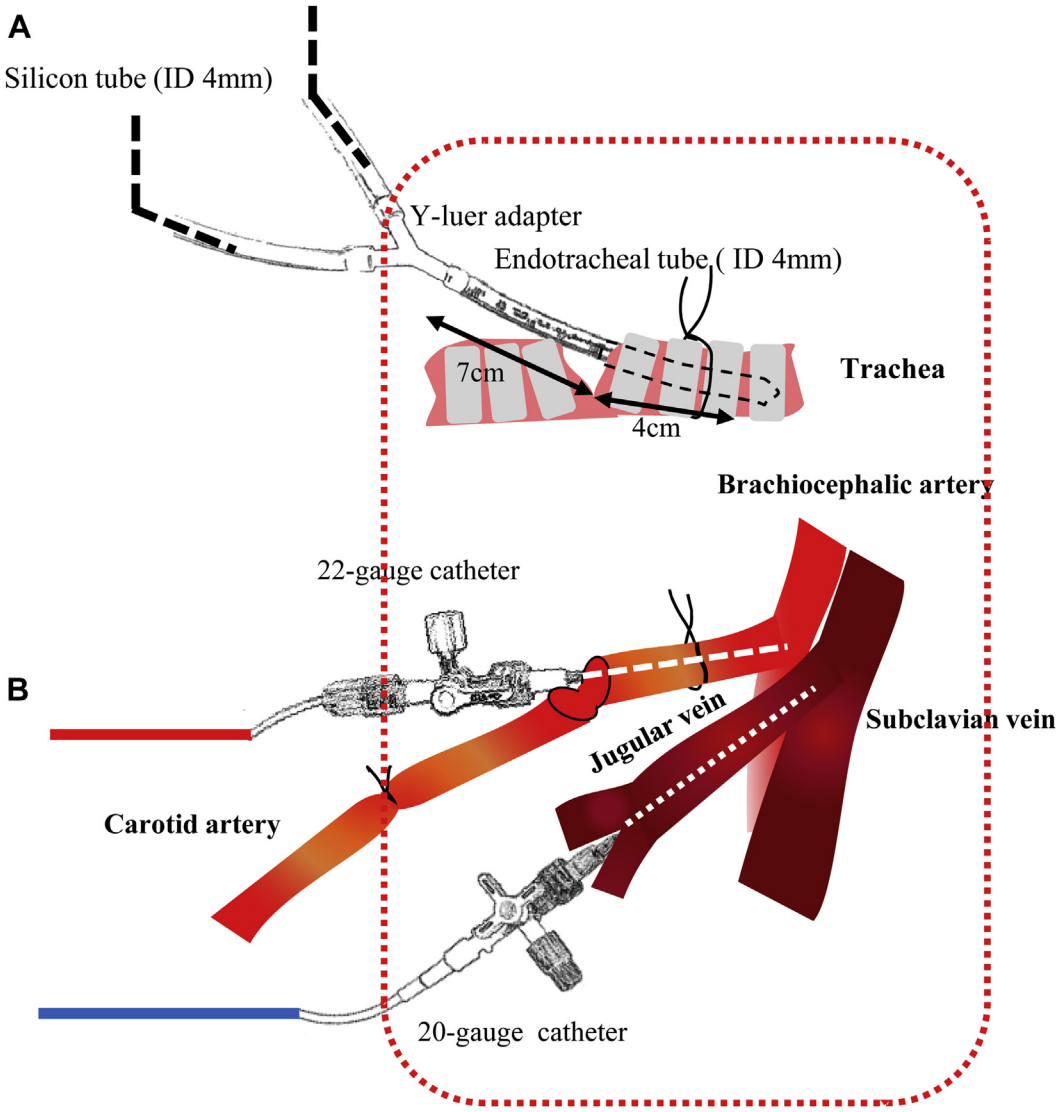


**Fig. 2 – Intratracheal intubation and cannulations into artery and vein. (A) intratracheal intubation. An endotracheal tube (internal diameter 4 mm) cut to 11 cm length is connected with Y-luer adapter. Silicon tube (internal diameter 4 mm is connected the ventilator and bifurcated Y-luer adapter. (B) Cannulations into artery and vein. The distal side of carotid artery is ligated. Cannulation into carotid artery is performed using 22-gauge catheter with cut down procedure. The tip of the catheter is placed at the origin of the carotid artery and catheter is ligated with artery from the outside. Cannulation into jugular vein is performed using 20-gauge catheter with puncture method. No ligation to keep venous flow. The tip of the catheter is placed at the joint portion of jugular vein and subclavian vein.**

filled with heparinized saline. We used the anatomic names of vessels described in the report of Zotti *et al.*[33]

### *Sample collection and experiment protocol*

Because the volume of samples necessary for a single BGA was 0.15 mL, we sampled the volume of 0.3 mL to allow for reanalysis. The total dead space of the catheter and three-way stopcock was 0.23 mL. We collected the samples, as follows: a volume of 0.5 mL (approximately twice the dead space) was removed; 0.3 mL of blood in a 1-mL heparin-rinsed syringe was sampled to measure the data. After sampling, 0.5 mL of blood in the "dead space" was retransfused, and 0.3 mL of heparinized saline was additionally infused to prevent coagulation. Michael *et al.*[34] recommend that blood sampling should be limited to 15% of total blood volume for 24 h to prevent a rapid change in the physiological conditions. Because they reported that the total blood volume of rabbits was 56 mL/kg body weight, we calculated the limit of sampling as 25.2 mL/d for rabbits weighing 3 kg.

All BGAs were examined by a portable blood gas analyzer (i-STAT; Abbott Point of Care Inc, Princeton, NJ) with a cartridge (CG4+; Abbott Point of Care Inc, Princeton, NJ). The i-STAT gas analyzer is able to measure the partial pressure of oxygen ($PO_2$), partial pressure of carbon dioxide ($PCO_2$), and pH of the whole blood. The oxygen saturation ($SO_2$) concentration

of bicarbonate ions ($HCO_3^-$) and the base excess of extracellular fluid (BEecf) were calculated by formulas (3-5).[35]

$$SaO_2 = 100 \times \left(X^3 + 150X/\left(X^3 + 150X + 23400\right)\right)$$

$$X = PO_2 \times 10^{\left(0.48(pH-7.4)-0.0013\left(HCO^{3-}-25\right)\right)} \quad (3)$$

$$Log(HCO_{3^-}) = pH + \log(PCO_2) - 7.608 \quad (4)$$

$$BEecf = (HCO_{3^-}) - 24.8 + 16.2(pH - 7.40) \quad (5)$$

We set two experiments to perform step-by-step analysis. In experiment 1, we aimed to study appropriate timing of arterial blood sampling. In experiment 2, we aimed to study appropriate timing of venous blood sampling and to study correlation between arterial and venous blood gas. To fit two goals, we separated two experiments to avoid excess blood loss.

### *Experiment 1: arterial BGA after RR change*

We changed the RR of the ventilator in seven rabbits, as indicated in Figure 3A. We defined steps 1-4 as follows. In steps 1 and 2, the RR phases were decreased from 40 per min to 30 per min and from 30 per min to 20 per min, respectively. Steps 3 and 4 were the recovery of phases from 20 per min to 30 per min and from 30 per min to 40 per min, respectively. We observed each step for 25 min.

According to previous reports in intensive care patients, the time to stabilization occurred 7-20 min after changing the respiratory condition.[36,37] Therefore, we set the observation time after intubation to 20 min with RR of 40 per min. The point at the time of 20 min observation was defined as "time 0," and blood sampling was started at this point. Table 1 summarizes the data of BGA of time 0 of step 1. Single arterial blood samples were collected at times 0.5, 1, 2, 3, 5, 10, 15, and 20 min after RR change in each step, and the BGA was examined (Fig. 3A). We used the value of 20 min in just the previous step as a substitute for the value of time 0. The total sample points were 33, and the total sample volume was 9.9 mL.

### *Experiment 2: arterial and venous BGA after the RR change*

We changed the RR as described in experiment 1 and performed a simultaneous sampling of the arterial and venous

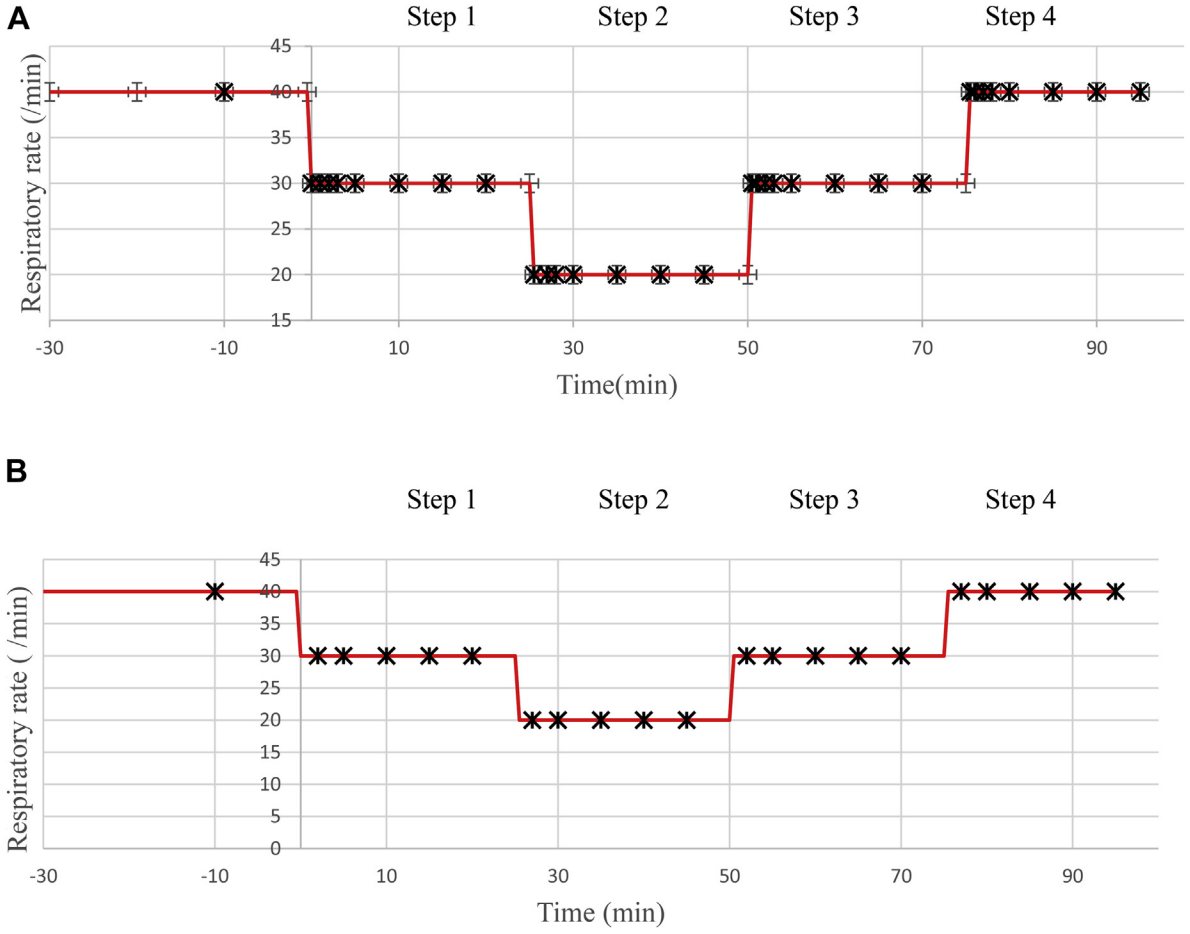


**Fig. 3 – Respiratory rate over time and point of blood sampling in the experiment. (A) Arterial blood gas analysis (experiment 1). The asterisk (*) shows the sampling point 20 min after artificial ventilation and 0.5, 1, 2, 3, 5, 10, 15, and 20 min after changing respiratory rate in every step. (B) Arterial and venous blood gas analysis (experiment 2). The asterisk (*) shows the sampling point 20 min after artificial ventilation and after 2, 5, 10, 15, and 20 min after changing the respiratory rate in every step. Respiratory rate in is 30 from 40 in step 1, 20 from 30 in step 2, 30 from 20 in step 3, and 40 from 30 in step 4. (Color version of figure is available online.)**

**Table 1 – Baseline of arterial blood gas analysis obtained after 20-min artificial ventilation with RR of 40 per min (mean ± standard deviation) $n = 14$.**

| $PaO_2$ (mm Hg) | $PaCO_2$ (mm Hg) | pH | $SaO_2$ (%) | $HCO_3^-$ (mmol/L) | BEecf (mmol/L) |
|---|---|---|---|---|---|
| 90.5 ± 7.12 | 30.36 ± 5.97 | 7.47 ± 0.093 | 97.7 ± 0.73 | 22.3 ± 4.06 | −1.57 ± 4.26 |

BEecf = base excess of extracellular fluid; $HCO_3^-$ = bicarbonate ions; $PaCO_2$ = arterial carbon dioxide partial pressure; $PaO_2$ = arterial oxygen partial pressure; RR = respiratory rate; $SaO_2$ = arterial oxygen saturation.

blood. Figure 3B shows the protocol and sample points of experiment 2. Using seven rabbits, we collected 147 points of simultaneous sampling of the arterial and venous blood gas (i.e., 294 samples in total, which included 147 arterial samples and 147 venous samples; 42 samples per rabbit). After 20 min of observation at an RR of 40 per min, we measured the BGA and value as time 0. The arterial and venous blood gases were measured at 2, 5, 10, 15, and 20 min for each step. The difference of sample timing from experiment 1 is due to technical limitation of the procedure. The total sample points were 21, and total sample volume was 12.6 mL. The BGAs were performed as soon as possible after sampling.

### *Statistical analysis*

In experiments 1 and 2, one-way repeated measures analysis of variance (i.e., one-way analysis of variance) was performed. The Tukey honest significant difference test was performed as a multiple comparison. We considered a "stable condition" by the following criteria: (1) a significant difference between the value at time 0 of each step and the value of the concerned point and (2) no significant difference between the values of the concerned point and the values of all subsequent points. In experiment 2, we additionally used regression analysis to assess the correlation of the arterial and venous data.

Statistical analyses were performed using commercial statistical software (Windows Excel Office 2013, Microsoft Co, Redmond, WA; and JMP Pro software, version 11.2.0, SAS Institute, Ltd, Cary, NC). Values of $P < 0.05$ were regarded as significant. Values are expressed as the mean ± standard deviation.

## Results

Fourteen rabbits were used in this study. Seven rabbits were used for arterial BGA (i.e., experiment 1), and seven rabbits were used for arterial and venous BGA (i.e., experiment 2). No rabbit died during the experiments. The data of arterial BGA at the starting point (i.e., RR = 40) are summarized in Table 1. The body weight of the 14 rabbits was 3.02 ± 0.09 kg. The results of experiments 1 and 2 are shown in Figures 4-6.

### *Experiment 1: arterial BGA after RR change*

Figure 4 presents the arterial $PO_2$ ($PaO_2$), pH, $PCO_2$ ($PaCO_2$), and $SO_2$ ($SaO_2$) after the RR change. The time to stabilization in each item after the RR change are summarized in Table 2. The $PaO_2$ and $SaO_2$ became stable in 0.5 min for all steps.

The $PaO_2$ decreased from 94.8 ± 7.2 mm Hg to 84 ± 16.2 mm Hg in 0.5 min ($P = 0.0030$ for time 0.5 min *versus* time 0) in step 1. After 0.5 min, the values were statistically the same. In step 2, the $PaO_2$ similarly decreased from 83.4 ± 8.9 mm Hg to 66.3 ± 6.9 mm Hg ($P < 0.0001$) in 0.5 min and became stable after 0.5 min. In step 3, the $PaO_2$ increased from 65.9 ± 9.2 mm Hg to 81.3 ± 10.3 mm Hg ($P < 0.0001$) in 0.5 min. In step 4, the $PaO_2$ increased from 82.1 ± 5.9 mm Hg to 98.1 ± 10.3 mm Hg ($P < 0.0001$) in 0.5 min.

In step 1, the $SaO_2$ decreased from 98.0% ± 0.6%, and the stable point was 96.0% ± 2.3% in 0.5 min ($P = 0.0124$). In step 2, the $SaO_2$ decreased from 96.1% ± 1.5% to 91.6% ± 3.1% ($P < 0.0001$) in 0.5 min and became stable. In steps 3 and 4, the $SaO_2$ increased from 90.3% ± 4.1% to 94.9% ± 2.0% ($P < 0.0001$) and from 95.7% ± 1.1% to 98.1% ± 0.7% ($P = 0.0001$), respectively, and became stable after 0.5 min.

The $PaCO_2$ also became stable within 3 min for all steps: it stabilized by 2 min in step 1, 1 min in step 2, 3 min in step 3, and 0.5 min in step 4. In step 1, the $PaCO_2$ increased from 29.1 ± 3.3 mm Hg to 32.1 ± 5.7 mm Hg ($P = 0.0006$) in 2 min and then became stable. In step 2, the $PaCO_2$ increased from 32.9 ± 4.9 mm Hg to 38.7 ± 6.3 mm Hg ($P = 0.0031$) in 1 min, and then became stable. In step 3, the $PaCO_2$ decreased from 40.3 ± 5.0 mm Hg to 40.4 ± 4.6 mm Hg ($P = 0.0103$) in 3 min. In step 4, the $PaCO_2$ decreased from 32.6 ± 3.6 mm Hg to 27.4 ± 0.2 mm Hg ($P = 0.0002$) in 0.5 min.

The pH became stable within 1 min in step 1, in 3 min in step 2, and in 1 min in step 3. In step 4, the pH did not become stable. In step 1, the pH decreased from 7.45 ± 0.05 to 7.41 ± 0.05 ($P = 0.0233$) in 1 min and then became stable. In step 2, the pH decreased from 7.40 ± 0.04 to 7.35 ± 0.05 ($P < 0.0001$) in 3 min and then became stable. In step 3, the pH also decreased from 7.34 ± 0.05 to 7.36 ± 0.04 ($P = 0.0409$) in 1 min. In step 4, the pH was 7.38 ± 0.04 at time 0, and thereafter it showed no statistically significant change.

### *Experiment 2: arterial and venous BGA after RR change*

Figure 5 shows the arterial and venous $PO_2$, pH, $PCO_2$, and $SO_2$ values. The venous oxygenation values changed with a smaller range compared with the arterial values. Table 2 summarizes the time to the stabilization of the venous and arterial $PO_2$, $PCO_2$, pH, and $SO_2$ after the RR change. The results of the venous BGA were not stable in most steps. With regard to the venous $PO_2$ ($PvO_2$) compared with the value of time 0, we could find no significant differences thereafter.

In step 1, the $PvO_2$ was 28.1 ± 4.8 mm Hg at time 0. The venous $SO_2$ ($SvO_2$) had a significant difference between time 0 and sporadic point, but it did not become stable. The $SvO_2$ moved irregularly between 49.4% ± 15.1% and 65.7% ± 7.6%.

The $PvCO_2$ became stable within 2 min in step 1 and within 15 min in step 2. The $PvCO_2$ increased from 37.3 ± 8.2 mm Hg

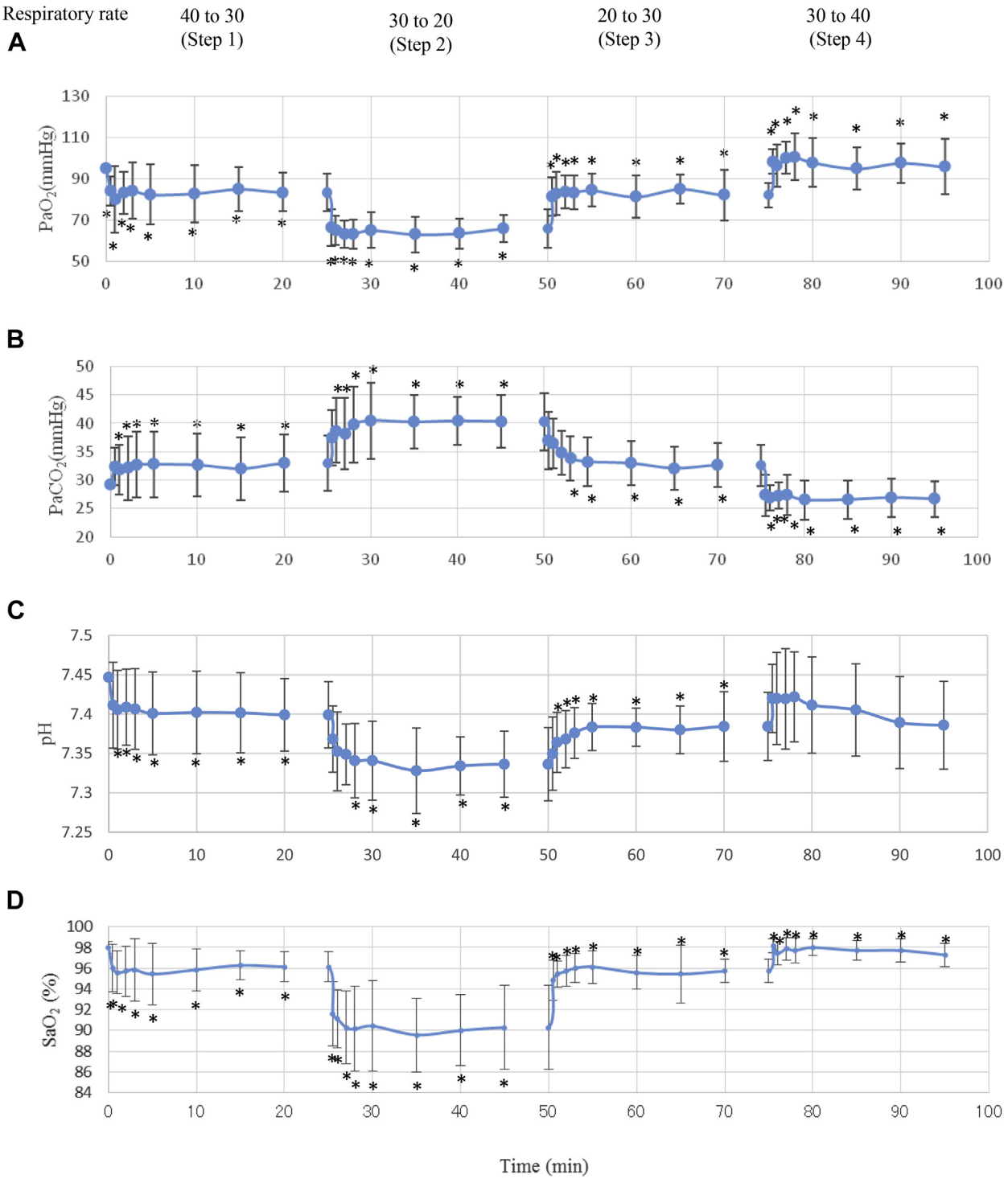


**Fig. 4 – Arterial blood gas analysis due to respiratory rate (experiment 1). (A) $PaO_2$, (B) $PaCO_2$, (C) pH, and (D) $SaO_2$. Values are presented as the mean ± the standard deviation. The asterisk (*) indicates that the value is significantly different in comparison to the initial value of the respiratory rate and is not significantly different in comparison to all subsequent values in the respiratory rate. (Color version of figure is available online.)**

to 42.0 ± 8.3 mm Hg in 2 min ($P = 0.0213$) in step 1 and increased from 44.2 ± 9.1 mm Hg to 51.4 ± 9.1 mm Hg ($P = 0.0028$) in 15 min in step 2. In steps 3 and 4, there were no significant differences between any points in multiple comparison.

In only step 1, the pH became stable within 5 min. The pH changed from 7.44 ± 0.11 to 7.39 ± 0.09 ($P = 0.0031$) in 5 min and then became stable. In step 2, the pH did not become stable at any point after 5 min in statistical multiple comparison. In steps 3 and 4, the pH did not change statistically

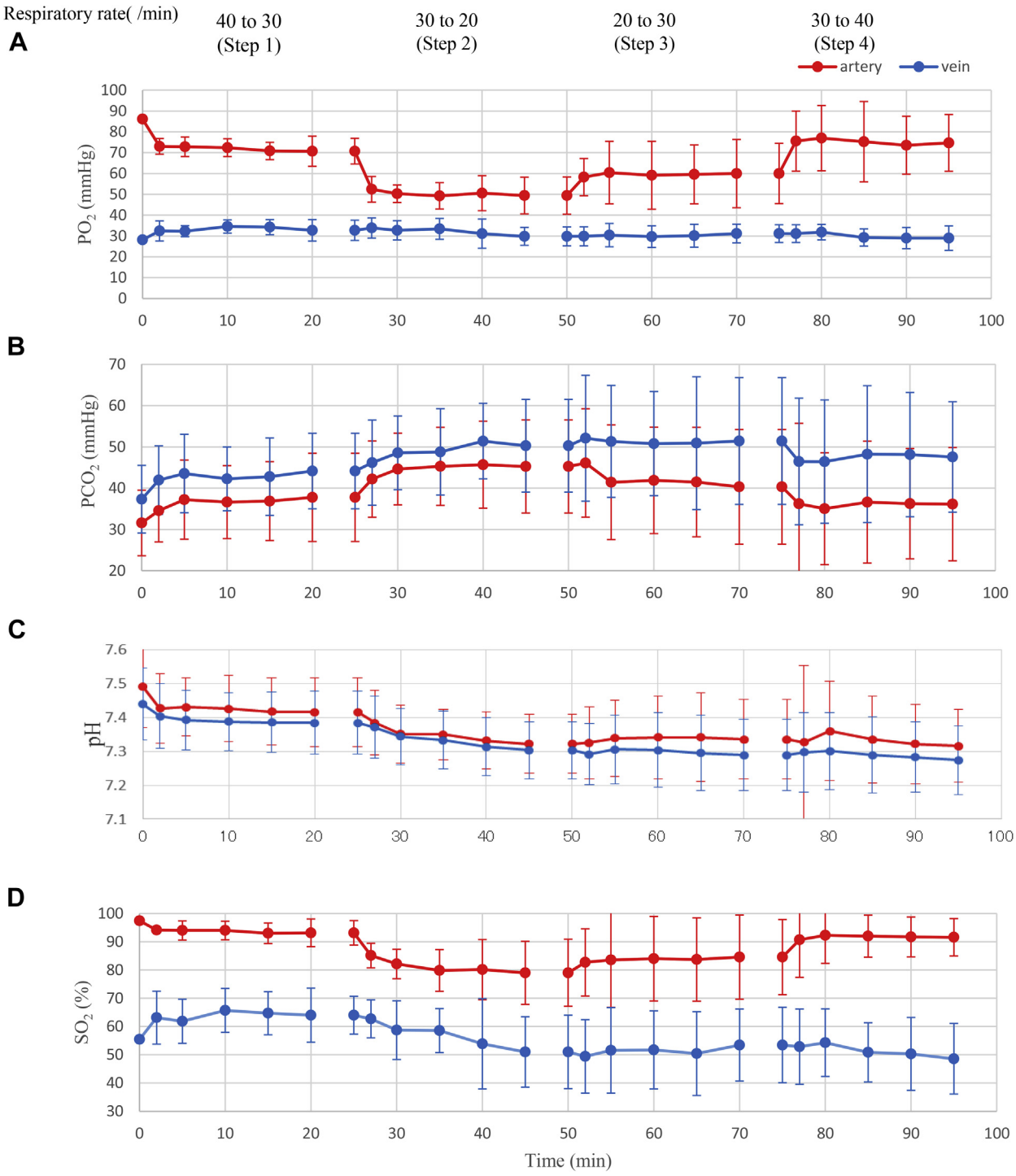


**Fig. 5 – Arterial and venous blood gas analysis with regard to the respiratory rate (experiment 2). (A) $PaO_2$, (B) $PaCO_2$, (C) pH, and (D) $SaO_2$. The red line shows the arterial blood gas analysis and blue line shows the venous values.**

from 7.30 ± 0.08 (time 0). In step 4, the pH also did not change statistically from 7.29 ± 0.10 (time 0).

The arterial and venous BGA, $PCO_2$, and pH changed in parallel. The correlation of the arterial and venous $PCO_2$ and the correlation of the arterial and venous pH are represented by the following equations (Fig. 6):

$$\text{Arterial } PCO_2 = 0.9316 \times \text{venous } PCO_2 - 4.4425$$

$$\text{Arterial pH} = 1.0835 \times \text{venous pH} - 0.5795$$

The correlation coefficients (R) were 0.9178 and 0.9453, respectively.

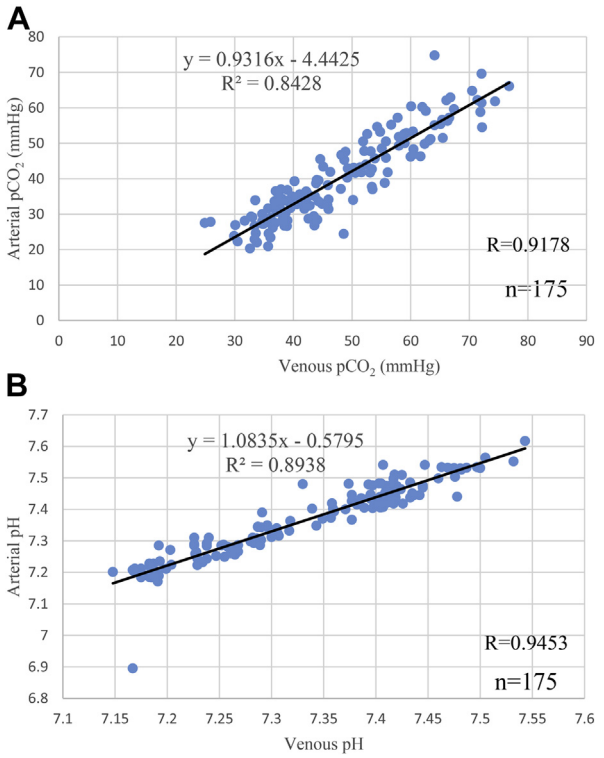


**Fig. 6 – Correlation between arterial and venous parameters (experiment 2). (A) Correlation between the arterial $pCO_2$ and venous $pCO_2$. (B) Correlation between the arterial pH and venous pH. (Color version of figure is available online.)**

**Table 2 – Time required to be stable in blood gas analysis (BGA) after changing respiratory rate (RR).**

| Respiratory rate (per min, step) | 40→30 (Step 1) | 30→20 (Step 2) | 20→30 (Step 3) | 30→40 (Step 4) |
|---|---|---|---|---|
| (A) Arterial BGA (experiment 1), sampling time: 0, 0.5, 1, 2, 3, 5, 10, 15, 20 min | | | | |
| $PaO_2$ | 0.5 | 0.5 | 0.5 | 0.5 |
| $PaCO_2$ | 1 | 1 | 3 | 0.5 |
| pH | 1 | 3 | 1 | Unstable |
| $SaO_2$ | 0.5 | 0.5 | 0.5 | 0.5 |
| (B) Venous BGA (experiment 2), sampling time: 0, 2, 5, 10, 15, 20 min | | | | |
| $PvO_2$ | Unstable | Unstable | Unstable | Unstable |
| $PvCO_2$ | 2 | 15 | Unstable | Unstable |
| pH | 5 | Unstable | Unstable | Unstable |
| $SvO_2$ | Unstable | 15 | Unstable | Unstable |

$PaCO_2$ = arterial carbon dioxide partial pressure; $PaO_2$ = arterial oxygen partial pressure; $PvCO_2$ = venous carbon dioxide partial pressure; $PvO_2$ = venous oxygen partial pressure; $SaO_2$ = arterial oxygen saturation; $SvO_2$ = venous $SO_2$.

## Discussion

In the arterial BGA (i.e., experiment 1), the $PaO_2$ became stable in 0.5 min for all steps. The $PaCO_2$ became stable in less than 3 min for all steps. The $SaO_2$ (which was calculated with the $PaO_2$, $PaCO_2$, and pH) also became stable in 0.5 min for all steps. The BEecf and $HCO_3^-$ did not become stable for all steps.

In experiment 2, we performed simultaneous sampling of the arterial and venous blood. The value of the venous sample did not become stable for most steps. The results of the arterial BGA in experiment 2 corresponded with the results of experiment 1. The value of the venous blood was different from that of the arterial blood, although we found significant positive correlations between the arterial and venous $PCO_2$ and pH ($r = 0.9178$ and $r = 0.9453$, respectively). The results of arterial BGA (i.e., experiment 1) showed that the $PaO_2$ and $SaO_2$ became stable in 0.5 min.

In experiment 1, the $PaO_2$ and $SaO_2$ became stable in shorter time than our hypothesis. We set the hypothesis based on the oxygenation equilibrium time in human and the cardiac output per kilogram body weight in rabbits. The oxygenation equilibrium time was obtained from patients in intensive care units with malfunctioning lung in some reports,[15,16] and we used animal with healthy lung. The time to stabilization that we obtained in this study might be shorter because our experiments were performed using healthy animal. Sasse *et al.*[36] described three main factors that could affect the equilibrium reaching time: (1) lung-blood interface of reflect ventilation-to-perfusion matching, (2) air movement into and out of the lungs, and (3) the mechanical transport of blood to the peripheral arteries. In our study, we used normal rabbits with the same body weight and ventilated them with a fixed tidal volume. Among these three factors, the lung interface and air movement were fixed throughout the experiment, and only the mechanical transport of blood to the peripheral arteries could affect the results.

In general, oxygen delivery in tissues is represented by the following equation:

$$\text{Oxygen delivery } (DO_2) = \text{cardiac output} \times \text{arterial oxygen content} \quad (6)$$

Rabbits have 2.39 times larger cardiac output per kilogram of body weight. Moreover, rabbits have a smaller distance between the heart and peripheral tissues and smaller vessel diameters than humans. Rabbits may have a more effective blood transport system in comparison to humans. This factor may be why the time to become stable was more than seven times shorter in rabbits.

Rabbits are widely used for experiments on the analysis of respiratory function such as validation of a pulse oximeter[12–14,20] because the hemoglobin of rabbits is optically similar to that of humans.[12,13] In several studies on BGA in rabbits,[12–14,20] investigators confirmed that the value of pulse oximeter are stabilized within 2 min. Therefore, 2 min are used for the timing of blood sampling. However, no rationale for 2 min was described, and therefore, the time of 2 min has been conventional. In our study, we determined that, after changing the RR, the time that the $PaO_2$ and $SaO_2$ became stable was 0.5 min for all steps.

The $PaCO_2$ became stable within 3 min, whereas the $PaO_2$ became stable in 0.5 min. The $PaO_2$ became stable in 0.5 min for all steps (Table 2), although the $PaCO_2$ did not mirror the

changes in the $PaO_2$. The $PaCO_2$ changed less than 10 mm Hg, whereas the $PaO_2$ changed more than 15 mm Hg (Fig. 4 and Table 2). The $PaCO_2$ may shift slower and range less than $PaO_2$. Ivanov and Nunn[38] reported that, after a rapid change in the tidal volume, the $PaCO_2$ moved slower than $PaO_2$. Our results showed the same trend. Slonim and Hamilton[39] also reported that the change in $PaCO_2$ did not mirror that of $PaO_2$ because $CO_2$ is generated in the process of metabolism. In our experiment, the $PCO_2$ gradually increased and increased further at the RR of 20 per min (step 2). In step 3, when we increased the RR to 30 per min, the $PaCO_2$ gradually decreased.

In the experiment 2, the value of venous BGA did not become stable in most steps. The pH and $PvO_2$ showed no change from time 0 for all steps. This result indicated that the oxygenation indexes, including $PO_2$, $SO_2$, and $PCO_2$ of venous blood, showed only a small change, even if the RR was reduced from 40 per min to 20 per min.

There was no correlation between the arterial and venous $PO_2$ and the arterial and venous $SO_2$ in our study. Korner *et al.*[40] report that acute hypoxia causes temporal tachycardia and subsequent bradycardia, and vasoconstriction raises blood pressure. This physiological response decreases cardiac output in hypoxia. Calbet *et al.*[41] and Mortola *et al.*[43] have shown that in acute hypoxia, oxygen delivery to the peripheral tissues decreased and the extraction of oxygen in the peripheral tissues increased little. Therefore, the consumption of oxygen in the tissues declined in hypoxia. In hypoxia, the dissociation curves of hemoglobin shifts to the right and the oxygen affinity of blood increases. This response maintains the effective oxygen delivery in low oxygen pressure.[44]

Oxygen consumption by tissues ($VO_2$) is defined by the following equation.[43]

$$\text{Cardiac output} = \frac{\text{VO2}}{\text{arterial oxygen content} - \text{venous oxygen content}} \tag{7}$$

$$\text{Oxygen Content in blood} = \left(1.34 \times \text{hemoglobin} \times \frac{\text{SaO2}}{100}\right) + 0.003 \times PaO_2 \tag{8}$$

Substitute (8) for (7),

$$VO_2 = \text{cardiac output} \times 1.34 \times \text{hemoglobin} \times \frac{\text{SaO2} - \text{SvO2}}{100} + 0.003 \times (PaO_2 - PvO_2) \tag{9}$$

Oxygen consumption is reflected by the gradient of arterial and venous value such as $PO_2$ and $SO_2$.

In our study, the gradient of the arterial and venous values such as $PO_2$ and $SO_2$ were the largest at the RR of 40 per min, and the differences were the smallest at the RR of 20 per min.

The $VO_2$ in Equation (9) decreased in hypoxia because the cardiac output and $SaO_2$–$SvO_2$ decreased. Calbet *et al.*[41] and Mortola *et al.*[42] described the decrease in oxygen consumption in hypoxic tissues and their findings supported these results.

To evaluate new medical devices such as NIRS that measure tissue oxygen saturation, the weighted average of oxygen saturation or partial pressure of an artery and vein in the same area are usually used as the standard.[9,44] In many studies,[43–45] the cervical artery and vein (which we used) are used as the sampling points to monitor cerebral oxygenation. The arterial blood oxygen saturation and partial oxygen pressure were used as the most reliable indexes for tissue oxygenation. The normal range of oxygenation indexes of arterial blood is relatively narrow, and the normal range is established, although the range of venous blood is much broader and the normal range is generally not established. This finding may be because the venous blood oxygenation is affected by many factors such as peripheral circulation, arterial blood oxygenation, and tissue metabolism; there are also considerable individual differences.[45] Watzman *et al.*[20] showed that the distribution of arterial and venous blood was different in different tissues. Thus, the normal range of oxygen saturation in each tissue has not been established and the difference from individual baseline should be assessed.[46] In our study, the individual difference of $PvO_2$ and $SvO_2$ were much larger than those of the artery. Additional studies should be performed to reveal the meanings and mechanisms of venous oxygenation. We sampled venous blood from the cervical vein, although the values of venous blood analysis were affected by the sample site, even if anatomically using the same central vein.[47] It should be important to define the exact site of blood sampling to evaluate the tissue oxygenation.

In experiment 2, the base-acid balance such as the pH and $PCO_2$ level showed a significant correlation between the arterial and venous values. Some studies[48–55] evaluated the indexes of acid-base balance, which includes the $PCO_2$, pH, $HCO_3^-$, and BEecf of the central venous blood, as a substitute for arterial BGA in shock patients. The results have been controversial; some studies[48–50] report that the venous value is an alternative to the arterial value, whereas some studies[51,52] report that the venous value is not to be considered or is to be considered as an alternative to the arterial value only in the normal condition.[53,54] In our study, although the venous pH and $PCO_2$ were different from the arterial values, a significant correlation was detected in the hypoxic condition.

Our experimental setting has several limitations. The first limitation was that we changed the RR at only three levels: 40 per min, 30 per min, and 20 per min. Thus, rabbits were exposed to relatively mild hypoxia. Therefore, we cannot discuss severe hypoxia. The second limitation was the limit of the observation time. We observed the rabbits for a maximum of 30 min for each step, after changing the RR; therefore, we

did not confirm whether the stability was maintained for a longer period.

Several studies[16,36] evaluated the equilibrium reaching time in intensive care patients with respiratory distress syndrome, and the most investigators changed the positive end-expiratory pressure or inspire-oxygen concentration. There are few studies about RR changes in our best knowledge. Baumgardner *et al.*[55] monitored $PaO_2$ in RR change using rabbits as a lung injury model, although they did not make reference to equilibrium time. We controlled the RR to produce hypoxia condition because RR is an important and the simplest ventilator setting. In our study, after changing the RR, the arterial blood gas value became stable within 0.5 min and remained stable until 20 min. In addition, the venous blood gas value did not become stable after changing the RR. We believe that our results establish a rationale for further experiments.

### *Conclusions*

We found that the arterial blood gas value became stable in 0.5 min after changing the RR, but the venous blood gas value did not become stable during 20 min. We also found that the indexes of the acid-base balance such as the pH and the arterial and venous $PCO_2$ showed different values but moved in parallel with significant correlation. Under limited conditions, the venous blood value may be used as an alternative to the arterial blood value only in defined equations.

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## Disclosure

Conflicts of interest: None.

REFERENCES

1. Vincent JL. Critical care—where have we been and where are we going? *Crit Care*. 2013;17:S2.
2. Bickler PE, Feiner JR, Severinghaus JW. Effect of skin pigmentation on pulse oximeter accuracy at low saturation. *Anesthesiology*. 2005;102:715–719.
3. Bohnhorst B, Peter CS, Poets CF. Pulse oximeters' reliability in detecting hypoxemia and bradycardia: comparison between a conventional and two new generation oximeters. *Crit Care Med*. 2000;28:1565–1568.
4. Eatwell K, Mancinelli E, Hedley J, et al. Use of arterial blood gas analysis as a superior method for evaluating respiratory function in pet rabbits (Oryctolagus cuniculus). *Vet Rec*. 2013;173:166.
5. Sood BG, McLaughlin K, Cortez J. Near-infrared spectroscopy: applications in neonates. *Semin Fetal Neonatal Med*. 2015;20:164–172.
6. Kanayama N, Niwayama M. Examiner's finger-mounted fetal tissue oximetry. *J Biomed Opt*. 2014;19:067008.
7. Salyer JW. Neonatal and pediatric pulse oximetry. *Respir Care*. 2003;48:386–398.
8. Dawson JA, Bastrenta P, Cavigioli F, et al. The precision and accuracy of Nellcor and Masimo oximeters at low oxygen saturations (70%) in newborn lambs. *Arch Dis Child Fetal Neonatal Ed*. 2014;99:F278–F281.
9. Solevåg AL, Dannevig I, Šaltytė-Benth J, Saugstad OD, Nakstad B. Reliability of pulse oximetry in hypoxic newborn pigs. *J Maternal Fetal Neonatal Med*. 2014;27:833–838.
10. Iyer P, McDougall P, Loughnan P, Mee RB, AI-Tawil KJ, Carlin J. Accuracy of pulse oximetry in hypothermic neonates and infants undergoing cardiac surgery. *Pediatr Crit Care*. 1996;24:507–511.
11. Garattini S. The necessity of animal experimentation. In: *The importance of animal experimentation for safety and biomedical research*. 1. Dordrecht, Netherlands: Kluwer Academic Publishers; 1990:1–4.
12. Vegfors M, Sjoberg F, Lindberg LG, Gustafsson U, Lennmarken C. Basic studies of pulse oximetry in a rabbit model. *Acta Anaesthesiol Scand*. 1991;35:596–599.
13. Vegfors M, Lindberg LG, Oberg PA, Lennmarken C. The accuracy of pulse oximetry at two haematocrit levels. *Acta Anaesthesiol Scand*. 1992;36:454–459.
14. Yang YX, Xie BS, Zhou ZX, Liu JN, Xue YY, Lv GL. Computer analysis system of blood oxygen saturation in an animal hypoxia model. *Med Biol Eng Comput*. 1998;36:355–358.
15. Byrad LG, Prince GA. Animal models of respiratory syncytial virus infection. *Clin Infect Dis*. 1997;25:1363–1368.
16. Hickling KG, Wright T, Laubscher K, et al. Extreme hypoventilation reduces ventilator-induced lung injury during ventilation with low positive end-expiratory pressure in saline-laveged rabbits. *Crit Care Med*. 1998;26:1690–1697.
17. Roughton FJW. *Transport of oxygen and carbon dioxide. In: Handbook of physiology. Section 3. Resipiration*. Vol. 1. Washington D.C.: American Physiological Society; 1964:767–826.
18. Edwards AWT, Korner PI, Thorburn GD. The cardiac output of the unanesthetized rabbit, and the effects of preliminary anaesthesia, environmental temperature and carotid occlusion. *Exp Physiol*. 1959;17:309–321.
19. Grossman W. Blood flow measurement: cardiac output and vascular resistance. In: *Grossman's Cardiac Catheterization, Angiography, and Intervention*. Vol. 1. Chapter 8. Alphen aan den Rijn, Netherlands: Wolters Kluwer Health; 2005.
20. Watzman HM, Kurth CD, Montenegro LM, Rome J, Steven JM, Nicolson SC. Arterial and venous contributions to near-infrared cerebral oximetry. *Anesthesiology*. 2000;93:947–953.
21. Matute-Bello G, Frevert CW, Martin TR. Animal models of acute lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2008;295:L379–L399.
22. Fischer B, Chavatte-Palmer P, Viebahn C, Santos AN, Duranthon V. Rabbit as a reproductive model for human health. *Reproduction*. 2012;144:1–10.

23. Lipman NS, Marini RP, Erdman SE. A comparison of ketamine/xylazine and ketamine/xylazine/acepromazine anesthesia in the rabbit. *Lab Anim Sci*. 1998;40:395–398.
24. Wenger S. Anesthesia and analgesia in rabbits and rodents. *J Exot Pet Med*. 2012;21:7–16.
25. Kim KS, Shim JC, Jun JH, Lee KH, Chung CW. Rabbits treated with chronic isepamicin are resistant to mivacurium and rocuronium. *Anesth Analg*. 1999;88:654–658.
26. Drorbough JE. Pulmonary function in different animals. *J Appl Physiol*. 1960;15:1069–1072.
27. Stahll WR. Scaling of respiratory variables in mammals. *J Appl Physiol*. 1967;22:453–460.
28. Lowry DW, Mirakhur RK, McCarthy GJ, Carroll MT, McCourt KC. Neuromuscular effects of rocuronium during sevoflurane, isoflurane, and intravenous anesthesia. *Anesth Analg*. 1998;87:936–940.
29. Terakawa Y, Ichinohe T, Kaneko Y. Rocuronium and vecuronium do not affect mandibular bone marrow and masseter muscular blood flow in rabbits. *J Oral Maxillofac Surg*. 2010;68:15–20.
30. Aeschbacher G, Webb AI. Propofol in rabbits. 2. Long-term anesthesia. *Lab Anim Sci*. 1993;43:328–335.
31. Chiu HM, Hirsh J, Yung WL, Regoeczi E, Gent M. Relationship between the anticoagulant and antithrombotic effects of heparin in experimental venous thrombosis. *Blood*. 1977;49:171–184.
32. Shah PS, Shah VS. *Continuous heparin infusion to prevent thrombosis and catheter occlusion in neonates with peripherally placed percutaneous central venous catheters (Review)*. Oxford, UK: The Cochrane Library; 2011. Issue 7.
33. Zotti A, Banzato T, Cozzi B. Cross-sectional anatomy of the rabbit neck and trunk: comparison of computed tomography and cadaver anatomy. *Res Vet Sci*. 2009;87:171–176.
34. Michael W. McGuill MW, Rowan AN. Biological effects of blood loss: Implications for sampling volumes and techniques. *ILAR NEWS*. 31;5–20:1989.
35. D'Orazio P. Blood gas and pH analysis and related measurements; approved guideline-Second edition. No. 8. In: *CLSI Guideline C46-A-2*. Vol. 29. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
36. Sasse SA, Jaffe MB, Chen PA, Voelker KG, Mahutte CK. Arterial oxygenation time after an FIO2 increase in mechanically ventilated patients. *Am J Respir Crit Care Med*. 1995;152:148–152.
37. Cakar N, Tugrul M, Demirarslan A, et al. Time required for partial pressure of arterial oxygen equilibration during mechanical ventilation after a step change in fractional inspired oxygen concentration. *Intensive Care Med*. 2001;27:655–659.
38. Ivanov SD, Nunn JF. Influence of the duration of hyperventilation on rise time of $PCO_2$ after step reduction of ventilation. *Respir Physiol*. 1968;5:243–249.
39. Slonim NB, Hamilton LH. Blood-gas transport. In: *Respiratory physiology*. 5th ed. **Chapter 8**. St. Louis: The C.V. Mosby Company; 1987.
40. Korner PI, Edwards AWT. The immediate effects of acute hypoxia on the heart rate, arterial pressure, cardiac output and ventilation of the unanaesthetized rabbit. *Exp Physiol*. 1960;16:113–122.
41. Calbet JA, Boushel R, Rådegran G, Søndergaard H, Wagner PD, Saltin B. Determinants of maximal oxygen uptake in severe acuter hypoxia. *Am J Physiol Regul Integr Comp Physiol*. 2003;284:R291–R303.
42. Mortola JP, Rezzonico R, Lanthier C. Ventilation and oxygen consumption during acute hypoxia in newborn mammals: a comparative analysis. *Respir Physiol*. 1989;78:31–43.
43. Kiening KL, Unterberg AW, Bardt TF, Schneider GH, Lanksch WR. Monitoring of cerebral oxygenation in patients with severe head injuries: brain tissue PO2 versus jugular vein oxygen saturation. *J Neuro Surg*. 1996;85:751–757.
44. Kreeger RN, Ramamoorthy C, Nicolson SC, et al. Evaluation of pediatric near-infrared cerebral oximeter for cardiac disease. *Ann Thorac Surg*. 2012:1527–1533.
45. Schell RM, Cole DJ. Cerebral monitoring: jugular venous oximetry. *Anesth Analg*. 2000;90:559–566.
46. Scheeren TWL, Schober P, Schwarte LA. Monitoring tissue oxygenation by near infrared spectroscopy (NIRS): background and current applications. *J Clin Monit Comput*. 2012;26:9348.
47. Agusti AG, Roca J, Barbera JA, Casademont J, Rodriguez-Roisin R, Wagner PD. Effect of sampling site on femoral venous blood gas values. *J Appl Physiol*. 1994;77:2018–2022.
48. Malatesha G, Singh NK, Bharija A, Rehani B, Goel A. Comparison of arterial and venous pH, bicarbonate, PCO2 and PO2 in initial emergency department assessment. *Emerg Med J*. 2007;24:569–571.
49. Kelly AM, McAlpine R, Kyle E. Agreement between bicarbonate measured on arterial and venous blood gases. *Emerg Med Australas*. 2004;16:407–409.
50. Middleton P, Kelly AM, Brown J, Robertson M. Agreement between arterial and central venous values for pH, bicarbonate, base excess, and lactate. *Emerg Med J*. 2006;23:622–624.
51. Razi E, Nasiri O, Akbari H, Razi A. Correlation of arterial blood gas measurements with venous blood gas values in mechanically ventilated patients. *Tanaffos*. 2012;11:30–35.
52. Gunes V, Atalan G. Comparison of ventral coccygeal arterial and jugular venous blood samples for pH, $pCO_2$, $HCO_3$, $Be_{ecf}$ and $ctCO_2$ values in calves with pulmonary diseases. *Res Vet Sci*. 2006;81:148–151.
53. Williams KB, Christmas AB, Heniford BT, Sing RF, Messick J. Arterial vs venous blood gas differences during hemorrhagic shock. *World J Crit Care Med*. 2014;3:55–60.
54. Malinoski DJ, Todd SR, Slone DS, Mullins RJ, Schreiber MA. Correlation of central venous and arterial blood gas measurements in mechanically ventilated trauma patients. *Arch Surg*. 2005;140:1122–1125.
55. Baumgardner JE, Markstaller K, Pfeiffer B, Doebrich M, Otto CM. Effects of respiratory rate, plateau pressure, and positive end-expiratory pressure on PaO2 oscillations after saline lavage. *Am J Respir Crit Care Med*. 2002;166:1556–1562.